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MITIGATIVE EFFECT OF FLAVONOID RICH FRACTION OF CYPERUS ROTUNDUS AGAINST SORBITOL DEHYDROGENASE AND ALDOLASE REDUCTASE

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ABSTRACT:

INTRODUCTION:

The nut grass plant, scientifically known as Cyperus rotundus L. and belonging to the Cyperaceae family, is a perennial herb with a history dating back to 2000 years in India. It has been widely utilised in Ayurvedic medicine to address various health issues. Over the past decade, numerous studies have provided evidence of its therapeutic properties, including its ability to alleviate pain, combat allergies, treat arthritis, fight candida infections, prevent dental caries, control seizures, alleviate diarrhoea, reduce nausea and vomiting, expel parasitic worms, manage histamine reactions, regulate blood sugar levels, lower blood pressure, reduce inflammation, combat malaria, address obesity, and as an antioxidant, among other benefits.

MATERIALS AND METHODS:

Preparation of Plant Extract:

The rhizomes of Cyperus rotundus collected were shade dried and then coarsely powdered. About six hundred grams of coarse material was weighed and packed in a Soxhlet extractor with 1000 ml of 70% hydro alcohol (70% ethanol and 30% water). Extraction was carried out using a hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a semi-solid material. The extracts were concentrated and used for further experiments.

RESULTS:

The flavonoid-rich fraction of Cyperus rotundus has demonstrated significant(p3) inhibition of Aldose Reductase, Sorbitol accumulation and Advanced Glycation end product,Mitigative effects against sorbitol dehydrogenase and aldolase reductase, enzymes involved in carbohydrate metabolism.

CONCLUSION:

Through our study we have concluded that Cyperus rotundus has the potential to inhibit AGEs and it's an natural alternative for Metformin (Antibiotic) the mitigative effect of the flavonoid- rich fraction of Cyperus rotundus against sorbitol dehydrogenase and aldolase reductase represents a promising avenue for the development of novel therapies.

Keywords: Cyperus rotundus, Aldolase reductase,Sorbitol dehydrogenase,Mitigative effect

INTRODUCTION

Cyperus rotundus L., commonly known as nutgrass and a member of the Cyperaceae family, is a perennial herb thought to have originated in India approximately 2000 years ago. It boasts a rich history in Ayurvedic medicine, where it has been employed to treat various health conditions(1). Over the past decade, multiple research studies have unveiled a plethora of beneficial attributes associated with this herb. These include its capacity to serve as an analgesic, alleviate allergies, combat arthritis, address candida infections, prevent dental cavities, manage convulsions, relieve diarrhoea, reduce nausea and vomiting, expel intestinal worms, control histamine responses,

regulate blood sugar levels, lower blood pressure, mitigate inflammation, combat malaria, tackle obesity, and act as an antioxidant, among other properties.(2)

Researchers have shown a keen interest in the flavonoid-enriched portion of Cyperus rotundus and its potential to inhibit sorbitol dehydrogenase and aldolase reductase enzymes. By elucidating the inhibitory capabilities of this fraction and delving into its mechanisms of action against these enzymes, there is optimism for the development of therapeutic applications in the management of diabetes and its associated complications.(3)

Cyperus rotundus stands as a versatile plant with a global history of use in traditional medicine for treating a wide array of ailments, such as stomach disorders, wounds, boils, and blisters (1-4). It has been the focus of numerous investigations that have spotlighted its diverse range of pharmacological and biological activities(4). These encompass its anti-Candida, anti-inflammatory, antidiabetic, antidiarrheal, cytoprotective, antimutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic and apoptotic, antipyretic, and analgesic properties(5). Previous examinations of the plant's chemical composition have identified alkaloids, flavonoids, tannins, starch, glycosides, furochromones, and a variety of novel sesquiterpenoids. This study aims to assess the potential mitigating effect of the flavonoid-rich fraction derived from Cyperus rotundus. Its primary objective is to explore whether this fraction can exert inhibitory effects on sorbitol dehydrogenase and aldolase reductase enzymes(6).

MATERIALS AND METHODS:

Plant Material:

The rhizomes of Cyperus rotundus were purchased from M/s. SKM Siddha and ayurvedic medicines India private limited, Tamil Nadu. The rhizomes were cleaned under running tap water and then shade dried at ambient temperature. Thereafter the dried rhizomes were pulverised into a coarse powder and ready for extraction.

Preparation of Plant Extract:

The rhizomes of Cyperus rotundus collected were shade dried and then coarsely powdered. About six hundred grams of coarse material was weighed and packed in a Soxhlet extractor with 1000 ml of 70% hydro alcohol (70% ethanol and 30% water). Extraction was carried out using a hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a semi-solid material. The extracts were concentrated and used for further experiments.

Chemicals and Reagents:

Aminoguanidine hydrochloride, Metformin and was procured from TCI Chemicals, India. DL-glyceraldehyde, D-glucose, Fructose, lithium sulphate, NADPH, NADP, dimethyl sulphoxide (DMSO), sorbitol, bovine serum albumin, perchloric acid, ammonium sulphate, Tris-HCl, EDTA, sucrose and sorbitol dehydrogenase were purchased from Sigma aldrich (St Louis, MO, USA). All other chemicals of analytical grade were obtained from Himedia, India and SRL chemicals, India(7).

Advanced Glycation end product (AGE) assay (Harris et al., 2011)

Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. The cyperus rotundus extract was evaluated for its activity on AGEs formation at different concentrations of 2.5-25µg/ml. AGE reaction mixture was constituted as follows; 1 mg/mL bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M glucose. The reaction mixture (2.75mL) was treated with different concentrations of cyperus rotundus (2.5-25µg/ml). Amino guanidine was used as positive control(8). After incubating at 37°C for 3 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelengths of 350 nm and 450 nm, respectively, using Biotek synergy multi-mode reader, USA. The percentage activity was calculated with respect to solvent control.

Determination of Aldose Reductase Inhibition (Reddy et al., 2011):

A total of 531µL of 0.1 M potassium buffer (pH 7.0), 90µL of NADPH solution (1.6 mM in potassium buffer), 90µL of recombinant human aldose reductase (AR) (6.5U/mg) (Sigma, USA - SRP6371-100UG), 90µL of ammonium sulphate solution (4 M in potassium buffer), and 90 µL of DL-glyceraldehyde (25 mM in potassium buffer) were mixed with 9µL of different concentrations of cyperus rotundus (2.5-25µg/ml) in a cuvette, and the activity of AR was assessed spectrophotometrically by measuring the decrease in NADPH absorbance at 340 nm for 3 min using a spectrophotometer (Biotek Synergy H4 multi mode reader, USA). Metformin was used as positive control. The inhibition of AR (%) was calculated using the following equation: $(1 - (\triangle A \text{ sample/min}) - (\triangle A \text{ blank/min})/(\triangle A \text{ control/min}) - (\triangle A \text{ blank/min})) \times 100\%$, where $\triangle A$ sample/min is the decrease in absorbance over 3 min with reaction solution, test sample, and substrate, and $\triangle A$ control/min without the test sample.

Sorbitol Accumulation Inhibition Assay (Malone et al., 1980):

5 ml of blood was collected into heparinized tubes from healthy volunteers after an overnight fast. The blood was immediately centrifuged at 2000 rpm for 5 min, 4ºC to separate the erythrocytes from the plasma. After discarding the plasma and buffy coat, add isotonic saline (0.9% NaCl) equal to twice the volume of the erythrocytes and centrifuged at 2000 rpm for 10min. Washed RBCs were suspended in Hank's balanced salt solution [HBSS] (pH 7.4) to a ratio of 1:10. Samples were incubated at 37°C for 3 h under normal (5.5mM) and high glucose (55mM) conditions. The effect of cyperus rotundus on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of hydroalcoholic extract of cyperus rotundus. At the end of incubation periods, RBC were centrifuged, washed with saline and again centrifuged. Red cell was precipitated with cold 6% perchloric acid to the ratio of 1:3. The homogenate was centrifuged at 2000 rpm at 4°C for 10 min and the pH of the supernatant was adjusted to 3.5 with 0.5M potassium carbonate. The sorbitol content of the supernatant was measured by fluorometric method. In brief, the reaction mixture contained the appropriate protein-free supernatant, 50mM glycine buffer (pH 9.4), 0.2mM NAD+, and 1.28U/ml sorbitol dehydrogenase. The mixture was incubated at 37°C for 30 min, and the relative fluorescence due to NADH was measured by a fluorescence spectrometer at an excitation wavelength of 366 nm and an emission wavelength of 452 nm.

STATISTICAL ANALYSIS:

Data were analysed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC50 values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standards groups using the Holm-Sidak Test.

RESULTS:

Fig 1: Advanced Glycation end product (AGE) assay

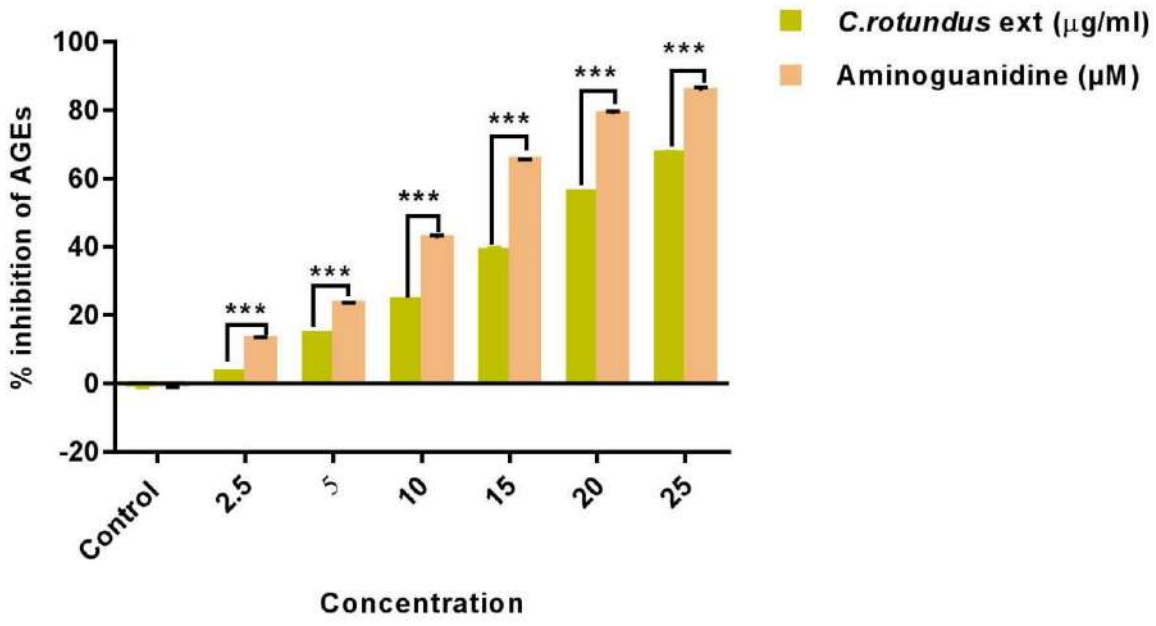


Fig 2:Aldose Reductase Inhibition assay

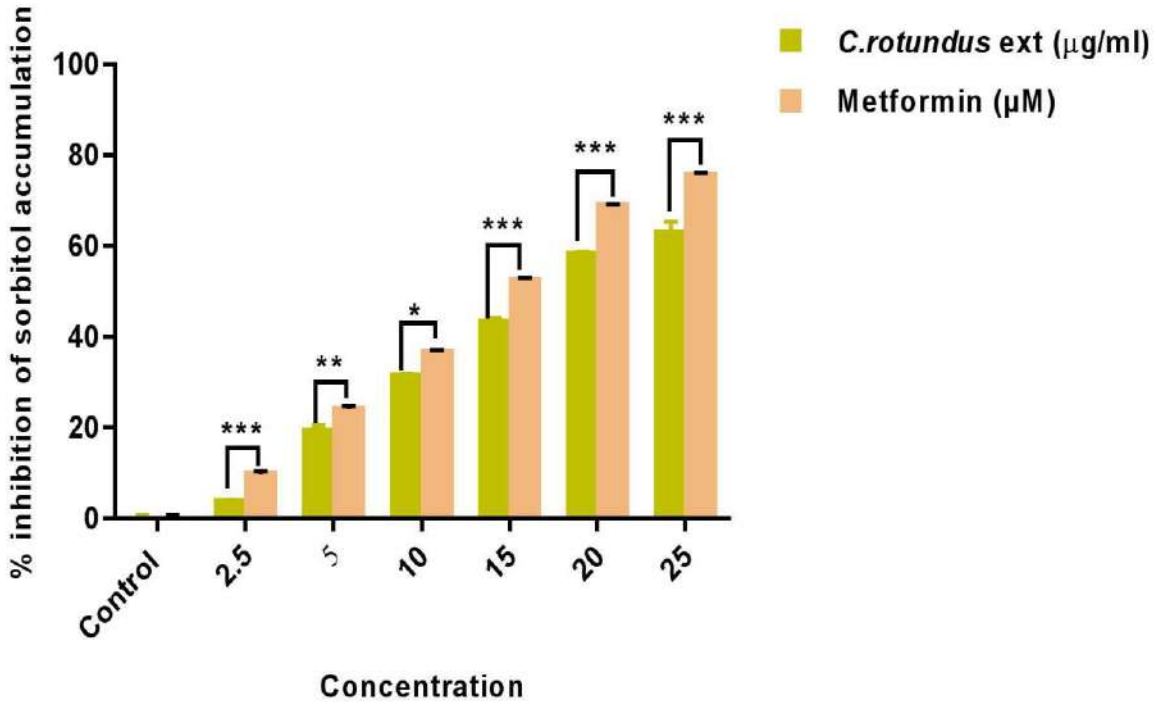
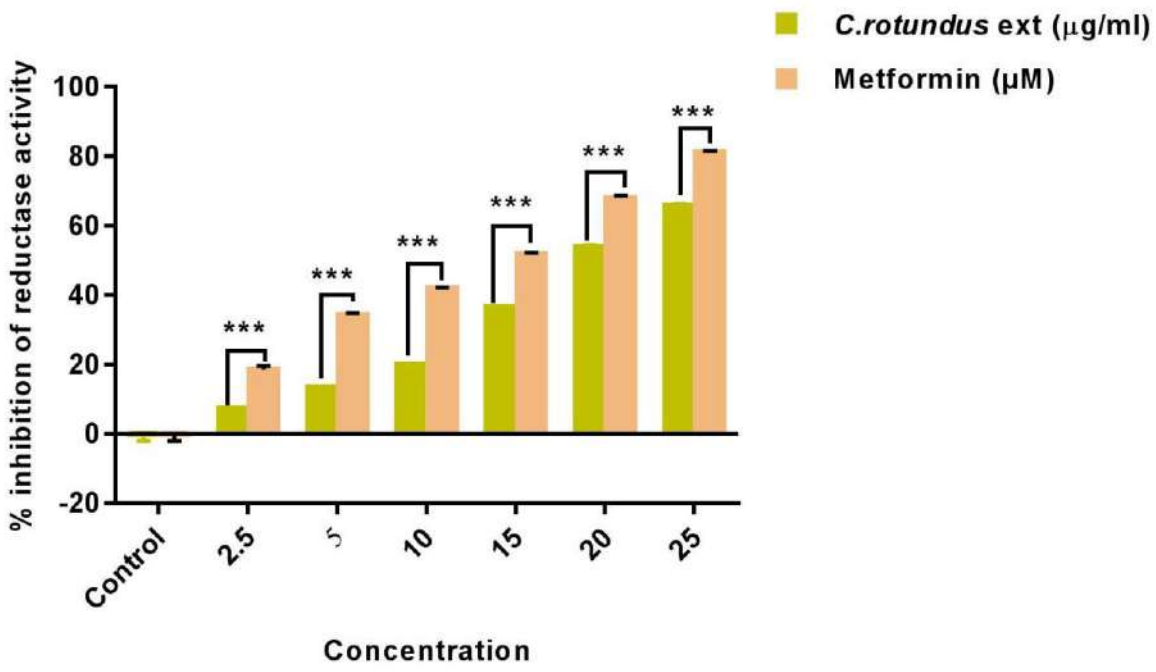


Fig 3 :Sorbitol accumulation inhibition assay



The flavonoid-rich fraction of Cyperus rotundus has demonstrated significant(p3) inhibition of Aldose Reductase, Sorbitol accumulation and Advanced Glycation end product Mitigative effects against sorbitol dehydrogenase and aldolase reductase, enzymes involved in carbohydrate metabolism. Dysregulation of these enzymes can contribute to the development and progression of metabolic disorders, particularly diabetes and its associated complications.

DISCUSSION

The flavonoid-rich fraction obtained from Cyperus rotundus has shown potential in mitigating the activity of two critical enzymes, sorbitol dehydrogenase and aldolase reductase, which play essential roles in glucose metabolism and certain metabolic disorders.

The available evidence suggests that the flavonoids in this extract can act as inhibitors of these enzymes, leading to a reduction in the conversion of sorbitol to fructose (a reaction catalysed by sorbitol dehydrogenase) and the conversion of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (catalysed by aldolase reductase). This inhibitory effect helps regulate the metabolic pathways involving these enzymes. Such modulation of enzymatic activities can be crucial in managing conditions where abnormal glucose metabolism is a concern. By preventing the accumulation of sorbitol, the extract may help mitigate complications associated with conditions like diabetes(6).

Similarly, inhibiting aldolase reductase can help control glycolytic flux and prevent the buildup of downstream metabolites linked to specific metabolic disorders. It's important to note that the current evidence is based on preliminary studies, and further comprehensive research, including in vivo and clinical investigations, is needed to validate the precise mechanisms and therapeutic potential of Cyperus rotundus's flavonoid-rich fraction in mitigating sorbitol dehydrogenase and aldolase reductase activity(9).

Regarding mutagenicity, it's worth noting that not all natural products and medicinal plants behave the same way. In the case of Cyperus rotundus, tests with various strains (TA98, TA100, TA1535, and TA1538) indicated that extracts from the plant's aerial parts did not increase the number of revertants, even at high concentrations, compared to spontaneous revertants. However, certain dilutions showed a statistically significant decrease in the number of revertants, suggesting a potential toxic effect(10).

Assessing the toxicity and mutagenicity of natural products and medicinal plants is crucial for safe usage. While the absence of mutagenicity in Cyperus rotundus extracts is promising, the observed toxic effects at specific dilutions warrant further investigation. More research is needed to comprehensively understand the pharmacological properties and safety profile of Cyperus rotundus and its extracts for medicinal purposes.The assay used to evaluate the Cyperus rotundus extract (CRE) measures its ability to protect deoxyribose from degradation by hydroxyl radicals. The reaction involves deoxyribose, which can be broken down by hydroxyl radicals. However, when CRE is present, it can scavenge hydroxyl radicals and inhibit the degradation of deoxyribose(11).

This assay can be conducted in two ways: with or without the addition of EDTA. EDTA is a chelating agent that can bind to metal ions like iron.(12) When the assay is performed without EDTA, it primarily assesses CRE's ability to scavenge hydroxyl radicals, which is a non-site-specific assay. On the other hand, when the assay includes EDTA, it evaluates the iron-chelating activity of CRE, which is referred to as a site-specific assay(13).

In the presence of hydroxyl radicals, deoxyribose breaks down into fragments, which can react with thiobarbituric acid (TBA) under low pH conditions to produce a pink colour. The extent to which the pink colour formation is inhibited by CRE indicates its capacity to scavenge hydroxyl radicals and/or chelate iron.(14) By analysing the degree of inhibition of deoxyribose degradation, researchers can gain insights into the hydroxyl radical scavenging and/or iron-chelating activities of the Cyperus rotundus extract. These activities are significant as they may contribute to the extract's potential antioxidant and therapeutic properties(15).

CONCLUSION

Through our study we have concluded that Cyperus rotundus has the potential to inhibit AGEs and it's an natural alternative for Metformin (Antibiotic) the mitigative effect of the flavonoid- rich fraction of Cyperus rotundus against sorbitol dehydrogenase and aldolase reductase represents a promising avenue for the development of novel therapies. The exploration of these natural compounds and their mechanisms of action may contribute to the advancement of treatment.

Conflict of interest:

The authors report no conflict of interest.The authors are alone responsible for the content and detailing of the paper

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Since it’s an in vitro study,ethical clearance is not required

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